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Procedia Food Science 1 (2011) 1099 – 1104

Procedia Food Science

11th International Congress on Engineering and Food (ICEF11)

Antemortem and postmortem biochemistry, drip loss and lipid oxidation of European sea bass muscle tissue

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Abstract

Slaughtering methods of farmed fish can influence swimming activity and stress levels with consequences for the flesh quality properties of fish fillets. For example, muscle metabolic activity prior to death can affect the concentration of oxygen, ATP and the pH of muscle. Handling stress of fish prior to harvesting is characterized by increased swimming activity which leads to anaerobic metabolism while energy stores are depleted and white muscle pH drops leading to acceleration of autolytic reactions after death with consequences for the organoleptic parameters of fish fillets and the filleting yield of farmed fish. In addition to the food quality aspects of the slaughtering method, the potential suffering of fish being handled during common aquaculture procedures or during slaughtering prompted scientific research in the welfare of farmed fish during harvesting. The purpose of this work was to investigate the effect of handing stress on post- mortem muscle pH and rigor mortis. European sea bass were harvested using two different levels of handling stress. Muscle pH was monitored shortly after death and after ice storage. Drip loss, and thiobarbituric acid reactive substances (TBARS) were measured in ice stored fillets. Antemortem stress resulted in rapid drop of muscle pH and onset of rigor mortis. Drip loss, and thiobarbituric acid reactive substances (TBARS) increased in the stressed fish. The results indicate that reducing handling stress during harvesting can delay rigor mortis and improve the quality of European sea bass fillets.

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

Keywords: fish quality; animal welfare; lipid oxidation

1. Introduction

Slaughtering methods of farmed fish can influence swimming activity and stress levels with consequences for the flesh quality properties of fish fillets. For example, muscle metabolic activity prior to death can affect the concentration of oxygen, ATP and the pH of muscle. Handling stress of fish prior

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Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

doi:10.1016/j.profoo.2011.09.164

to harvesting is characterized by increased swimming activity which leads to anaerobic metabolism while energy stores are depleted and white muscle pH drops leading to acceleration of autolytic reactions after death with consequences for the organoleptic parameters of fish fillets and the filleting yield of farmed fish [1]. In addition to the food quality aspects of the slaughtering method, the potential suffering of fish being handled during common aquaculture procedures or during slaughtering prompted scientific research in the welfare of farmed fish during harvesting [16]. The purpose of this work was to investigate the effect of handing stress on post- mortem muscle pH and rigor mortis of sea bass. Handling stress during slaughtering can be reduced with the application of anaesthetic. Traditionally used anaesthetics in aquaculture include tricaine methanesulphonate (MS-222), benzocaine, etomidate, metomidate, 2-phenoxyethanol and quinaldine. In farmed fih, MS-222 appeared to be the most widely used anaesthetic. However, recently there has been considerable interest in another fish anaesthetic, clove oil extracted from the clove tree, *Eugenia caryophyllus* (syn. *Syzygium aromaticum*) which is generally considered a reasonable alternative of fish anaesthetic with low cost and of no risk to human health [12,18].

2. Materials and Methods

Sea cage cultured European sea bass juvemiles (BW 171,1 g ±21,82) were maintained for two weeks in holding tanks with 19,8 °C ± 1 °C water temperature. Water oxygen saturation was maintained above 80%.

The fish were subjected to two different methods of slaughtering. In one tank (High handling stress group, HHSG) the water was lowered and the fish were captured using a net and the fish were killed by immersion in and ice cold bath (4 part ice:1part sea water). In the other tank (Lower handling stress group, LHSG) the level of water was lowered and fish were anaesthetized moderately by immersion in a 30mg l^{-1} clove oil bath [10,12] for 5 minutes. Subsequently the fish were slaughtered by immersion in ice cold sea water (4 part ice: 1 part sea water). After 30 min, the fish were stored with the ventral side upwards in ice. After 30 min, the fish were stored with the ventral side upwards in ice. Body temperature of the fish was determined at regular intervals using a temperature probe (HI 145, Hanna Instruments).

Rigor mortis index was estimated by measuring the sag of the tail when a fish is placed with the front half of its body on a horizontal surface. The rigor mortis index (%) was estimated by the following equation [3,9]:

$$RI = [(h0 - h)/h0]*100 \quad (1)$$

where *ho* and *h* is the initial and final sag of the tail respectively.

Twenty fillets were dissected from fish of each group. The fillets were patted dry, weighed, individually wrapped in polyethylene bags, and stored on ice. Fillet percent drip-loss (DR%)during storage was calculated according to the following equation: DR% = [(initial fillet weight−final fillet weight) / initial fillet weight]*100.

About two hours after harvesting, epaxial white muscle (10-20g) was homogenized with in 1-2ml of double distilled water. The pH was estimated in duplicates with a glass pH electrode (HI 2210, Hanna Instruments). Lactate levels in homogenised muscle tissue were measured according to Barnett and Pankhurst [2].

Lipid oxidation was measured colorimetrically [9] according to the thiobarbituric acic reactive substances (TBARS). Samples of sea bass white mauscle (0.5 g) was homogenized in a solution of KCl 6.5 g L^{-1} and 1.5 mL of Tris-maleate, 80 m M, pH 7.4., Subsequently 0.5mL of 2 mM ascorbic acid was added and the samples were incubated at 37 °C for 30 min to induce lipid peroxidation. After the incubation, 3 mL of 0.7 M HCl and 3 mL of 0.05 M thiobarbituric acid (TBA) was added and the samples were incubated at 95 ^{0}C for 20 min and subsequently the samples were cooled and briefly centrifuged at 2000 rpm and 2 mL of 200 g L^{-1} TCA was added and the absorbance at 530nm was measured. A standard

solution with malondialdehyde was used to obtain a calibration curve and the levels of TBARS were expressed as mg of malondialdehyde (MDA) per kg of fish muscle.

Significance of differences was assessed with ANOVA. Data were arc–sin transformed prior to statistical analysis.

3. Results and Discussion

The skeletal muscle tissue of fish, including the European sea bass, contains muscle fibres with different histochemical and biochemical properties [7, 17]. The red muscle fibres form a superficial layer which extends deeper into the myotome but they constitute a minor portion of the skeletal muscle. The white muscle fibers, which constitute the bulk of muscle tissue are used to support burst swimming and are characterised by anaerobic metabolism. An intermediate layer of pink muscle fibers is located between the two layers. [11, 17]. In this study, we monitored biochemical changes in the white muscle of farmed sea bass which were slaughter with different procedures. As white muscle tissue constitutes the majority of fish fillets, we hypothesised that biochemical parameters of this tissue, would accurately reflect the post-harvest changes in fillet biochemistry of fish.

Table 1 . Progress of anaesthesia in sea bass. LHSG: Clove oil treated fish; HHSG: High handling stress group. Stage 0: fully active fish, Stage 5: No response to external stimuli & no breathing

TIME (minutes) after immersion in ice chilled water bath	**Body Temperature (^{0}C)**	**Stage of Anaesthesia**	
		HHSG (Control)	LHSG (clove oil)
0	20	0	0
3	5,6	3	4
6	3,9	4	5
12	3,1	5	5

Table 2. Drip loss and levels of Thiobarbituric acid reactive substances (TBARs) after 12 days ice storage of sea bass. LHSG: Clove oil treated fish; HHSG: High handling stress group. n=10 per group, Average values (+/- s.d.).

	LHSG(Clove oil treated fish)	**HHSG (Control)**	**ANOVA**
Fillet Drip loss (%)	4.77 (0.48)	5.39 (0.52)	P=0.006
TBARS	1.04 (0.07)	1.16 (0.02)	*
(μg MDA mg–1)			P=0,001)

The different methods of slaughtering used in the present work varied in terms of the anticipated level and duration of stress. The fish of the HHSG exhibited significantly longer period of escape swimming activity during the immersion in an ice cold bath. This can be explained by the considerable time which is required for the body temperature of fish immersed in ice slurry to drop below 5^{o}C and be fully lethargic (Figure 1, Table 1). Naturally, this problem was not observed in the moderate anaesthetised fish (LHSG) during the immersion in the ice slurry. The results indicate that the intensity of handling stress during slaughtering of sea bass can influence the development of biochemical changes and the onset of rigor mortis (Figure 2). The levels of lactate present in the muscle tissue of the two groups varied according to the killing method, the lactate levels of the HHSG fish were higher (11.64±1.08 μmoles g^{-1}) than the

LHSG fish (9.6±1.1 μmoles g^{-1}). This difference in lactate levels was also reflected in the muscle pH (Figure 3) and it indicates a higher level of slaughtering stress in the HHSG. Under handling stress, fish exhibit an escape swimming behaviour, which utilises the anaerobic metabolic pathways of the fast white skeletal muscle which lead to increased lactate.

Our results support the hypothesis that increased levels of handling stress can lead to increased lipid oxidation in the fillets of the harvested fish [5]. The handling stress prior to slaughtering results in rapid ATP depletion and reduced nucleotides such as NAD+ and NADP+ which are involved in the regeneration of various pro-oxidant substances [6] may have contributed in the difference of lipid oxidation between the stressed group (HHSG) and the clove oil treated fish. Lipid oxidation of fish fillets is highly undesirable with negative consequences for the organoleptic and the nutritional parameters of the fillets. The clove oil treated fish exhibited lower rate of lipid oxidation and drip loss during storage. In agreement with previously works, on sea bass and sea bream [1] the result indicate that a reduction of slaughtering stress can reduce the suffering of farmed fish during harvesting and also improve the quality of the fillets [14,16] during storage, with benefits for both the animals and the consumers.

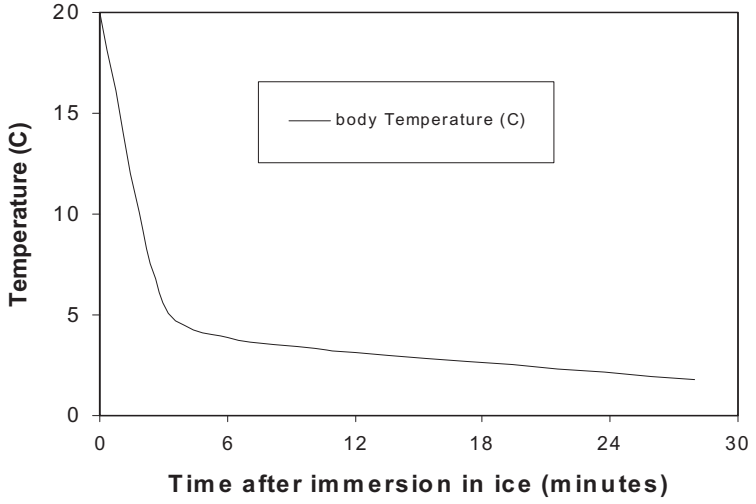

Fig. 1. Post-mortem body temperature drop during ice-storage of ice-killed slaughter sea bass

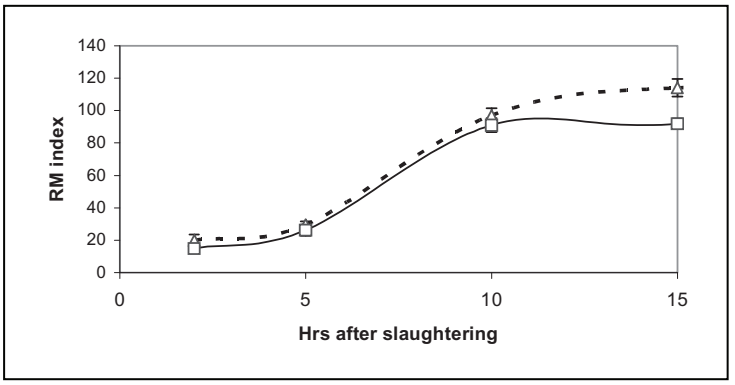

Fig. 2. Post-mortem development of rigor mortis index of the HHSG(triangles, dotted line) and LHSG (squares, solid line) slaughtered sea bass

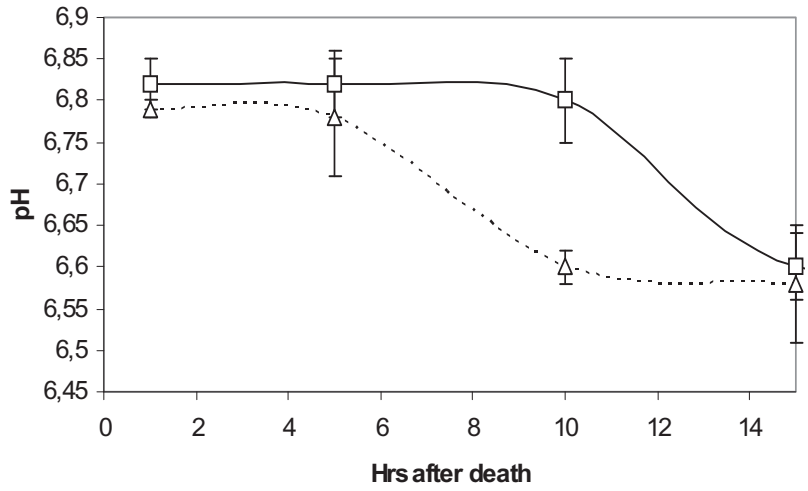

Fig. 3. Post-mortem drop of pH during ice-storage of the HHSG(triangles, dotted line) and LHSG (squares, solid line) slaughtered sea bass

4. Conclusion

Our results indicate the significance of slaughtering procedure for both the food quality aspects and the welfare of the harvested farmed fish. In the commonly used ice-slurry slaughtering method, the death of the un-anaesthetized fish of the HHSG appeared to progress much slower than the clove oil treated fish. This long agony of the HHSG appears to be a less humane killing method with consequences for the meat quality of the harvested fish.

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Presented at ICEF11 (May 22-26, 2011 – Athens, Greece) as paper MFS179.